Acta Medica Okayama

Volume 46, *Issue* 5 1992 *Article* 3

OCTOBER 1992

Hepatitis C virus antibody titration in patients with chronic hepatitis C, before and after interferon treatment.

Michiko Takahashi, *Okayama University*
Gotaro Yamada, *Okayama University*
Rieko Miyamoto, *Okayama University*
Toshihiko Doi, *Okayama University*
Hisashi Endo, *Okayama University*
Hiroshi Nishimoto, *Okayama University*
Shigeatsu Fujiki, *Okayama University*
Hiroyuki Shimomura, *Okayama University*
Motowo Mizuno, *Okayama University*
Takao Tsuji, *Okayama University*

Hepatitis C virus antibody titration in patients with chronic hepatitis C, before and after interferon treatment.*

Michiko Takahashi, Gotaro Yamada, Rieko Miyamoto, Toshihiko Doi, Hisashi Endo, Hiroshi Nishimoto, Shigeatsu Fujiki, Hiroyuki Shimomura, Motowo Mizuno, and Takao Tsuji

Abstract

We measured hepatitis C virus antibody titers in 13 patients with chronic hepatitis C to determine whether titration of hepatitis C virus antibody was useful or not, to predict and evaluate the efficacy of interferon (IFN) treatment. During administration of IFN, hepatitis C virus titers declined in all patients. Antibody titers performed before treatment as well as just at the end of treatment did not correlate with change of the alanine aminotransferase levels during administration of IFN. Antibody titers declined continuously after treatment in 5 patients with normal alanine amino-transferase levels for over 6 months after discontinuation of IFN. Antibody titers rose again in 6 patients whose alanine aminotransferase levels fluctuated after treatment. An exceptional pattern of change occurred in 2 patients whose antibody titers declined continuously although their alanine aminotransferase levels fluctuated after treatment. Repeated titration of hepatitis C virus antibody appears to be useful for evaluating the long-term efficacy of IFN treatment.

KEYWORDS: titiration of hepatitis C virus antibody, interferon, chronic hepatitis C, efficacy of treatment

---

*PMID: 1279945 [PubMed - indexed for MEDLINE]

Acta Med Okayama 46 (5) 331-336 (1992)

Hepatitis C Virus Antibody Titration in Patients with Chronic Hepatitis C, Before and After Interferon Treatment

Michiko Takahashi*, Gotaro Yamada, Rieko Miyamoto, Toshihiko Doi, Hisashi Endo, Hiroshi Nishimoto, Shigeatsu Fujiki, Hiroyuki Shimomura, Motowo Mizuno and Takao Tsuji

First Department of Internal Medicine, Okayama University Medical School, Okayama 700, Japan

We measured hepatitis C virus antibody titers in 13 patients with chronic hepatitis C to determine whether titration of hepatitis C virus antibody was useful or not, to predict and evaluate the efficacy of interferon (IFN) treatment. During administration of IFN, hepatitis C virus titers declined in all patients. Antibody titers performed before treatment as well as just at the end of treatment did not correlate with change of the alanine aminotransferase levels during administration of IFN. Antibody titers declined continuously after treatment in 5 patients with normal alanine aminotransferase levels for over 6 months after discontinuation of IFN. Antibody titers rose again in 6 patients whose alanine aminotransferase levels fluctuated after treatment. An exceptional pattern of change occurred in 2 patients whose antibody titers declined continuously although their alanine aminotransferase levels fluctuated after treatment. Repeated titration of hepatitis C virus antibody appears to be useful for evaluating the long-term efficacy of IFN treatment.

Key words : titration of hepatitis C virus antibody, interferon, chronic hepatitis C, efficacy of treatment

Since the test for antibody to hepatitis C virus (anti-HCV) was developed (1), several researchers have titrated anti-HCV by applying the assay and have suggested that anti-HCV titers declined, accompanied by the progression of chronic liver disease type C (2), or that anti-HCV values declined and became negative in patients with continuously normalized alanine aminotransferase (ALT) levels after treatment with interferon (IFN) (3). Recently, a method to detect hepatitis C virus RNA (HCV-RNA) has been developed (4). However, this assay is not convenient as a routine test, and measuring the amount of hepatitis C virus (HCV) is still difficult. In this study, we titrated anti-HCV in patients with chronic hepatitis C to determine whether the titration of anti-HCV was useful or not to predict or evaluate the efficacy of IFN treatment.

* To whom correspondence should be addressed.

Subjects and Methods

Patients. All 13 patients with chronic hepatitis C (8 men and 5 women, 19-62 years of age) examined in this study had persistently elevated ALT values for at least 1 year. The diagnosis of chronic hepatitis C was

confirmed histologically by liver biopsy, and virologically by the presence of positive anti-HCV, negative HBsAg, and low titers or the absence of titers of anti-HBc. The presumed source of infection was a blood transfusion in 4 patients. Duration from blood transfusion to treatment of IFN was 1.2 to 31 years. Known duration of hepatitis in the patients that had not been transfused was 1–27 years.

Treatment. Patients were treated with IFN-alpha or IFN-beta. Eight patients had a dose of 3 mega units (MU) everyday for 4 weeks, 1 patient had a dose of 9 MU everyday for 8 weeks, and 4 patients had a dose of 3 or 6 MU three times a week for 12 or 24 weeks.

The value of ALT was measured semimonthly during administration of IFN, then once a month to evaluate the efficacy of the treatment. Antibody to HCV was tested using a commercially available kit: Ortho HCV Ab ELISA test kit (Ortho Diagnostic Systems Inc., Raritan, NJ, USA). Hepatitis C virus RNA was detected by a reverse transcription-polymerase chain reaction, using the 5'-noncoding region as the primer (5).

Statistics. Results were compared by means of Wilcoxon's U test. When the probability (P) was over 0.05, results were judged to be insignificant (NS).

Results

Optical density (OD) values of anti-HCV in serum samples of two patients, which were diluted to various concentrations are shown in Fig. 1. Dilution of serum which also diluted anti-HCV, correlated well with OD values from 0.300 to 3.000, and the following relationship was found.

$$\text{Log (rate of dilution)} = -4.10\times10^{-1}\,(\text{tested OD value}) + a$$

a: constant

Optical density values of most anti-HCV-positive samples were over 3.000 when measured without dilution. The samples were appropriately diluted so that OD values might be 0.300–3.000 when measured. Optical density value (titer) was expressed as OD value in undiluted serum. Actually titer was calculated by applying the following formula.

$$\text{OD value (titer)} = \frac{\log(\text{rate of dilution})}{4.10\times10^{-1}} + \text{tested OD value}$$

ALT levels before treatment were 65–440 IU/l (Table 1). Liver biopsy specimens were classified as chronic persistent hepatitis (4 patients), chronic active hepatitis; moderately active (3 patients), chronic active hepatitis; severely active (4 patients), and liver cirrhosis (2 patients).

ALT levels at the end of treatment declined to normal in 7 patients, and in 3 of them ALT levels continued to be in normal range after discontinuation of IFN. In 2 of 6 patients who did not have normalization of ALT levels during administration of IFN, ALT levels gradually decreased and normalized after the end of treatment.

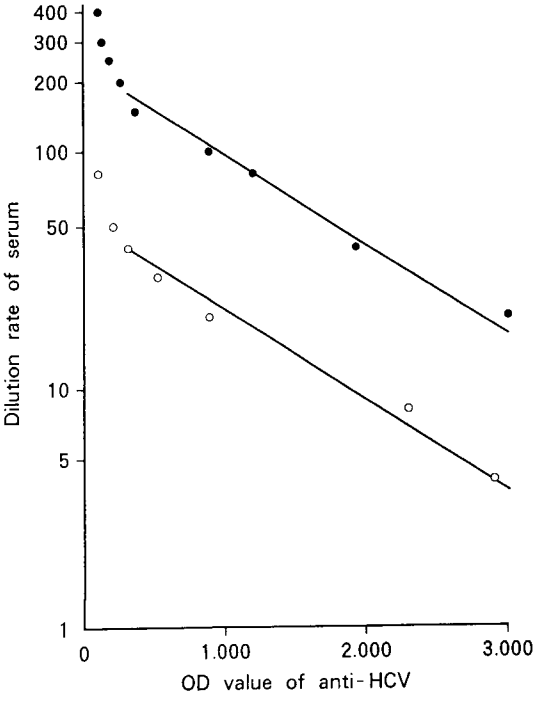

Fig. 1 Relationship between serum dilution and optical density value. Serum samples from two patients with chronic hepatitis C who had high anti-HCV titers were diluted, and tested for anti-HCV. ●: patient E.Y.; ○: patient H.D.

The mean OD value of anti-HCV before treatment was 4.961 (range, 1.157–7.787) in 7 patients with normal ALT levels during treatment, and 3.787 (2.785–5.220) in 6 patients without normal ALT levels during treatment. The OD values of pretreatment in the 5 patients with normal ALT levels after treatment were 5.597, 5.439, 4.349, 3.663, and 2.785.

In all 13 patients, the OD values decreased during IFN therapy. The average decrease between OD values before treatment and OD values at the end of treatment was 15 % (range, 1 %–30 %) in patients who experienced normalized ALT levels during treatment, and in those without normalized ALT levels during treatment it was 9 % (1 %–19 %). There was no significant correlation between the percentage of change of titer during treatment and response of ALT during treatment. The decrease in OD values in the patients with normalization of ALT levels over 6 months after treatment were 16 %, 2 %, 1 %, 4 % and 4 %.

In 6 of the 13 patients, OD values of anti-HCV rose again after treatment accompanied with a fluctuation in ALT levels (Fig. 2). One typical case with re-elevated anti-HCV titer (Case 5) is presented in Fig. 3. The 7 patients with continuous decreases in OD values consisted of the 5 with the normalization of ALT levels for over 6 months after treatment, and 2 with continuously

Table 1 Change of optical density value of anti-HCV and HCV-RNA before and after interferon treatment

Case No.	Histological diagnosis	Treatment (weeks)	ALT (IU/l)				Response to IFN		OD value of anti-HCV				HCV-RNA		
			Pre	Post	6M	12M	During	After	Pre	Post	6M	12M	Pre	Post	6M
1	CPH	12	72	13	11	7	R	R	5.597	4.718 (16)	3.630 〔23〕	ND	+	−	−
2	LC	12	105	17	13	12	R	R	5.439	5.317 (2)	3.946 〔26〕	ND	+	−	−
3	CAH 2A	4	262	26	7	7	R	R	4.349	4.322 (1)	2.699 〔38〕	2.184 〔49〕	+	−	−
4	CPH	4	109	30	106	119	R	N	3.238	2.985 (8)	3.303 〔−11〕	3.440 〔−15〕	ND		
5	CPH	4	169	25	435	195	R	N	7.787	5.892 (24)	7.158 〔−21〕	ND			
6	CPH	24	440	15	63	178	R	N	7.158	5.033 (30)	7.178 〔−43〕	ND			
7	CAH 2A	4	86	25	124	103	R	N	1.157	0.871 (25)	ND	2.580 〔−196〕			
8	CAH 2B	4	288	355	36	25	N	R	3.663	3.499 (4)	3.220 〔8〕	2.974 〔15〕	+	−	±
9	CAH 2B	4	123	99	24	20	N	R	2.785	2.681 (4)	0.459 〔83〕	0.202 〔92〕	+	−	−
10	CAH 2A	24	195	45	136	120	N	N	2.918	2.584 (11)	2.819 〔−9〕	ND	ND		
11	CAH 2B	8	145	75	117	101	N	N	4.156	3.351 (19)	3.314 〔1〕	ND			
12	LC	4	65	38	65	171	N	N	5.220	5.178 (1)	5.192 〔−3〕	5.476 〔−6〕			
13	CAH 2B	4	95	105	89	100	N	N	3.980	3.443 (13)	2.960 〔14〕	2.727 〔21〕			

Pre: before IFN treatment; Post: at the end of IFN treatment; During: during administration of IFN; After: after the end of IFN; R: normalized ALT levels; N: fluctuated ALT levels; ( ): reduction rate (%) of OD value between before treatment and at the end of treatment; 〔 〕: reduction rate (%) of OD value between at the end of treatment and 6 months or 12 months after treatment; CPH: chronic persistent hepatitis; CAH 2A: chronic active hepatitis, activity moderate; CAH 2B: chronic active hepatitis, activity severe; LC: liver cirrhosis; ND: not done

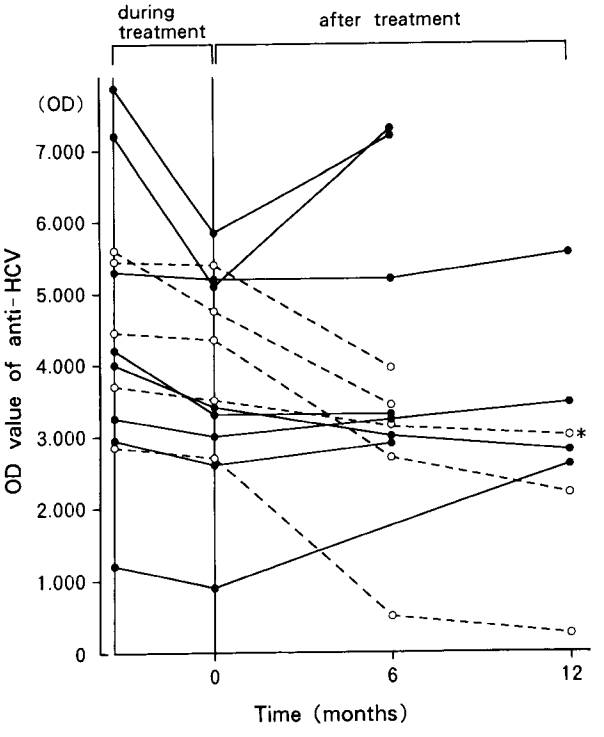

Fig. 2 Change in optical density value of anti-HCV before and after interferon treatment. OD values of patients with normalized ○, and fluctuating ●, ALT levels over 6 months after IFN treatment, *: patient with positive HCV-RNA at 6 months after IFN treatment regardless of normalization of ALT levels over 6 months.

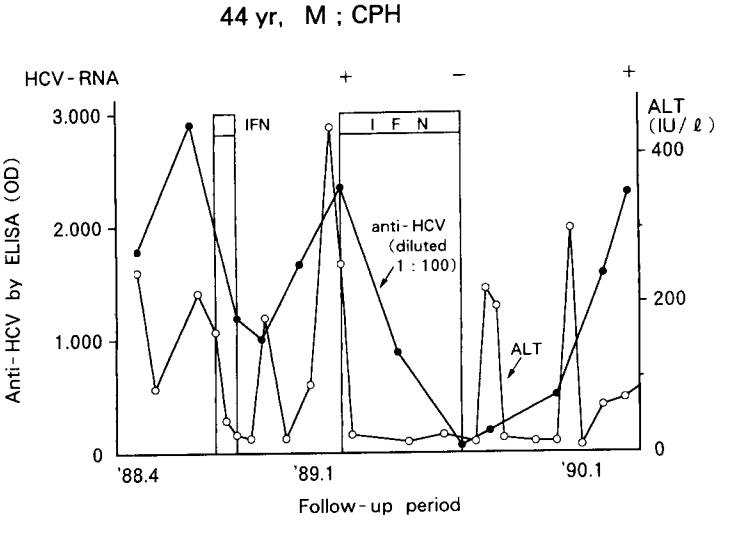

Fig. 3 Clinical course of a patient with fluctuating anti-HCV titer. CPH: chronic persistent hepatitis

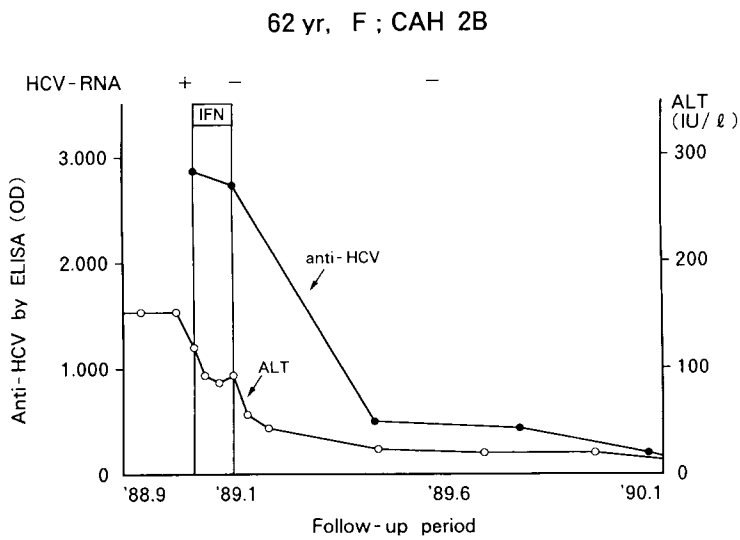

Fig. 4 Clinical course of a patient with disappearance of anti-HCV after interferon treatment. CAH 2B: chronic active hepatitis, activity severe

fluctuating ALT levels after treatment. Among the 5 patients with normal ALT levels for over 6 months, the patients with negative HCV-RNA at 6 months after treatment had larger decreases in OD values than the 1 patient without negative HCV-RNA. One patient with a sustained response and negative anti-HCV 8 months after treatment (Case 9) is shown in Fig. 4. The 2 patients with continuously fluctuating ALT levels after treatment had decrease of anti-HCV titers 6 and/or 12 months after treatment. However, the decreases in OD values were small, compared with the 4 patients with both normal ALT levels for over 6 months and continuously negative HCV-RNA.

Discussion

Our results demonstrated that the titers of anti-HCV in pre-treated patients had no correlation with normalization of elevated ALT values during IFN administration. Furthermore, in 5 patients who had normal levels of ALT for over 6 months after the discontinuation of IFN, their anti-HCV titers before treatment were widely distributed and it seemed that anti-HCV titers before treatment also had no correlation with the long-term outcome.

Variable decreases in anti-HCV titers during treatment were seen in all patients, not correlated with response of ALT levels during treatment. However, OD values of anti-HCV re-elevated in 6 of 8 patients whose ALT levels fluctuated after treatment. Conversely, in the 5 patients with normalized ALT levels for over 6 months after the end of IFN, OD values continuously decreased. This is identical with the finding reported by Kumada *et al.* (3). In the 2 patients with declines of OD values unaccompanied by normalized ALT levels after treatment, and the 1 patient with continued normal ALT levels for over 6 months after treatment unaccompanied by disappearance of HCV-RNA, the reduction of OD values after treatment was small. When anti-HCV was titrated for these patients after treat-

ment, the change of anti-HCV titers was likely to correlate with ALT levels or serum HCV-RNA after treatment.

We reported previously that there were a few patients in whom normal ALT levels continued for 1-2 years and more after IFN treatment ended, and yet, reactivation of hepatitis was observed (6). It was supposed that the patient with positive HCV-RNA in spite of the continuation of normal ALT levels might experience a reactivation of hepatitis, and a careful follow up was required.

It has been shown that the major action of IFN in chronic hepatitis C was antiviral (7-9). Accordingly, monitoring the amount of HCV-RNA should be useful for evaluating the efficacy of IFN treatment. However the assay of HCV-RNA remains inconvenient as a clinical test. Tanaka *et al.* reported that the presence of anti-HCV was closely related to the long-term prognosis in patients with acute and chronic hepatitis C (10), and titers of anti-HCV were higher in serum samples of patients with chronic evolution of acute hepatitis than in those with resolving acute hepatitis acquired from transfusion (11). Furthermore, nonstructural region 3 (NS3) of the HCV genome, that serves as the anti-HCV kit antigen, seems to be related to helicase which is essential for RNA replication (12). Accordingly, it is probable that the existence of anti-HCV indicates viremia. Although direct measurment of HCV-RNA or HCV antigen itself is the best way to examine the presence of HCV, the assays are still not convenient for clinical use. In our study, it has been strongly suggested that the titration of anti-HCV provides a good indicator to evaluate the long-term effects of IFN treatment in patients with hepatitis C.

References

1. Kuo G, Choo Q-L, Alter HJ, Gitnick GL, Redeker AG, Purcell RH, Miyamura T, Dienstag JL, Alter MJ, Stevens CE, Tegtmeier GE, Bonino F, Colombo M, Lee WS, Kuo C, Berger K, Shuster JR, Overby LR, Bradley DW and Houghton M: An assay for circulating antibody to a major etiologic virus of human non-A, non-B hepatitis. Science (1989) **244**, 362-364.
2. Iino S and Hino K: Diagnosis of hepatitis C. J Clin Sci (1990) **26**, 1526-1533 (in Japanese).
3. Kumada H, Arase Y, Chayama K, Saitoh S, Ikeda K, Matsumoto T, Sakai Y, Kobayashi M and Unakami M: The usefulness of HCV antibody titers in interferon therapy. Acta Hepatol Jpn (1990) **31**, 902-906 (in Japanese).
4. Weiner AJ, Kuo G, Bradley DW, Bonio F, Giorgio S, Lee C, Rosenblatt J, Choo Q-L and Houghton M: Detection of hepatitis C viral sequences in non-A, non-B hepatitis. Lancet (1990) **335**, 1-3.
5. Okamoto H, Okada S, Sugiyama Y, Tanaka T, Sugai Y, Akahane Y, Machida A, Mishiro S, Yoshizawa H, Miyakawa Y and Mayumi M: Detection of hepatitis C virus RNA by a two-stage polymerase chain reaction with pairs of primers deduced from the 5'-noncoding region. Jpn J Exp Med (1990) **60**, 215-222.
6. Takahashi M, Yamada G, Doi T, Endo H, Nishimoto H and Tsuji T: Analysis of liver histology of chronic non-A, non-B hepatitis treated with interferon. Acta Hepatol Jpn (1992) **33**, 1-8 (in Japanese).
7. Kato N, Yokosuka O, Omata M, Hosoda K, Imazeki F, Tagawa M and Ohto M: The relationship between the amount of hepatitis C virus quantified by competitive RT-PCR (CRT-PCR) and the effect of interferon treatment. Acta Hepatol Jpn (1991) **32**, 76-77 (in Japanese).
8. Chayama K, Saitoh S, Arase Y, Ikeda K, Matsumoto T, Sakai Y, Kobayashi M, Unakami M, Morinaga T and Kumada H: Effect of interferon administration on serum hepatitis C virus RNA in patients with chronic hepatitis C. Hepatology (1991) **13**, 1040-1043.
9. Brillanti S. Garson JA, Tuke PW, Ring C, Briggs M, Masci C, Barbara L and Tedder RS: Effect of alpha-interferon therapy on hepatitis C viraemia in community-acquired chronic non-A, non-B hepatitis. J Med Virol (1991) **34**, 136-141.
10. Tanaka E, Kiyosawa K, Sodeyama T, Nakano Y, Yoshizawa K, Hayata T, Shimizu S, Nakatsuji Y, Koike Y and Furuta S: Significance of antibody to hepatitis C virus in Japanese patients with viral hepatitis. J Med Virol (1991) **33**, 117-122.
11. Tanaka E, Kiyosawa K, Sodeyama T, Nakano Y, Shimizu S, Seki T, Furuta S and Kameko M: Anti-HCV and long-term prognosis in patients with non-A, non-B post-transfusion hepatitis. Jpn J Gastroenterol (1990) **87**, 1003-1009 (in Japanese).
12. Chambers TJ, Hahn CS, Galler R and Rice CM: Flavivirus genome organization, expression, and replication. Annu Res Microbiol (1990) **44**, 649-688.

Received January 29, 1992; accepted May 28, 1992.